Review of Coconut “Lethal Yellowing” type diseases
Diversity, variability and diagnosis

Abstract: Coconut palms (Cocos nucifera L.) can be affected by several types of Lethal Yellowing (LY) diseases worldwide. Some of the syndromes are caused by phytoplasmas, small bacteria that are impossible to detect by light microscopy. Amplification of a given gene of the phytoplasmas by polymerase chain reaction (PCR) is the most convenient diagnosis method. The problem is that there are at least 28 “groups” of phytoplasmas and only one pair of primers -P1/P7- commonly used for PCR. As these primers belong to a very conserved gene, false positives are frequent. Consequently, alternative primers specific to one “strain” (or subgroup) have to be used, such as LY-F/LY-R for the Caribbean LY, Rohde primers for LD Tanzania. Such specific primers are sometimes restrictive. Indeed, there is variability within each strain and the sequence of the primers has to be adapted to that variability. There are at least five LY subgroups. The subgroups can only be identified by restriction fragment length polymorphism or sequencing. In Africa, two subgroups of LY phytoplasmas have been identified so far.

Key words: coconut, Lethal Yellowing, Cape Saint Paul Wilt, diversity, variability, phytoplasma, PCR, 16S rDNA, syndrome, Ghana, Mozambique, Tanzania

In the 1830s, a disease resembling what is known today in the Caribbean as Coconut Lethal Yellowing (LY) was rife in the Cayman Islands [1, 2]. Since then, this type of LY disease has spread in the Caribbean region and central America. The same type of disease was observed on coconut palms in Tanzania, in East Africa, at the beginning of the 20th century [3, 4], then in West Africa, in Togo and Ghana [5]. The Caribbean, from Florida to Honduras, and tropical Africa, are currently the zones affected by these Lethal Yellowing Type Syndromes (LYTS). Indeed, one has to refer to a syndrome – development of successive symptoms – to describe these diseases. Like many other monocots, the coconut palm only displays a limited number of symptoms in response to biotic and abiotic stresses. Seeing a coconut palm with yellow fronds during a one-off inspection does not necessarily mean LY. Many mineral deficiencies cause fronds to turn yellow. Likewise, the existence of coconut stems that have lost all their fronds can have various causes. Lastly, it needs to be known that, in the Caribbean zone, two other coconut diseases, Hartrot caused by a trypanosomatid – Phytoponas sp. – [6, 7] and Red Ring, caused by the nematode Bursaphelenchus cocophilus Cobb. [8] can be confused with LY, even after observation of the syndrome.

In this review, we shall focus on the various LYTS to which phytoplasmas (e.g., Mycoplasma-like-organisms [MLO]) have been linked, either under the electron microscope, or by polymerase chain reaction (PCR).

Symptomatology
The very first symptom in LYTS involving phytoplasmas, commonly called LY in most cases, is for all the nuts to fall, both ripe and unripe. The existence of nuts of all sizes and all ages at the foot of a coconut palm is the first alarm signal. It is only after the nuts have fallen that leaflets start to turn yellow at the tip of a lower frond among the oldest. The yellowing spreads towards the stem. Right from the yellowing lower frond stage, blackening can be seen – necrosis – on the rachillas of a newly opened inflorescence. If the next inflorescence is collected, still wrapped in its spathe, and opened, browning can be seen on some or all of the male flowers, which may be detached from their rachillas, and on the tips of the rachillas themselves.

Rotting of the spear (f0), then of the immediately younger fronds (f-1, f-2) occurs more or less rapidly, before the yellowing reaches the youngest fronds. The yellow fronds then turn brown, dry out, hang down the stem, and eventually fall. In the end, only the stem remains, terminating in five to six young yellow fronds that are smaller than normal. This type of “tuft” eventually snaps due to the gradual spreading of the rot throughout the meristem zone after the spear has shown signs of necrosis, or following a gust of wind.

It should be noted that the Hartrot syndrome in the Caribbean zone exhibits exactly the same stages. The reason probably lies in the fact that each of these diseases is caused by a micro-organism that multiplies in the sap, in the phloem sieve tubes.

Distribution of LY type syndromes
Caribbean – America
It is in the Caribbean – the West Indies – that LYTS were reported for the first time at the end of the 20th century [1, 2]. It can be imagined that the disease spread naturally from island to island in that zone: Cayman Islands-Jamaica-Cuba-Haiti-Dominican Republic, as those islands are close to each other. It may also be that humans played a role, as short distances are conducive to trade.

In 1969, LY was detected in Key Largo – northwest part of the Florida Keys archipelago – then it spread step by step as far as Palm Beach county [9]. However, it may be that LY reached Florida via the southern tip of the Keys (Key-West) near to Cuba as early as the 1930s [9].

The west coast of Florida has remained relatively unscathed, mainly due to serious epidemiological monitoring, combined with rapid containment measures (felling, insecticide treatments and antibiotic treatments). It was then, not until the very beginning of the 1980s, that LY took a real lead to the tip of the Yucatan peninsula in Mexico. In this case, the hypothesis most often put forward is human introduction of infectious vectors from Florida. In fact, that period corresponds to the hotel development of the future seaside resort in Cancun. Uncontrolled imports of planting material from Florida (palms and grasses for golf course greens – grasses that are propitious to vector development) may have enabled the introduction of infectious vectors. From the Yucatan tip, the disease gradually decimated the coconut plantations as far as Vera Cruz. Southwards it spread to Belize. It was not until the beginning of the 1990s that LY was identified on the island of Roatan located off La Ceiba in Honduras, Central America [10]. The disease, which rapidly passed over to the continent, spread as far as Guatemala to the West and to Colon in the East where it stopped spreading eastwards. For the moment, it has not affected Nicaragua or El Salvador. Two explanations have been put forward for this other leap by LY: either it was a similar process to that assumed for Mexico, or it was by the passive transportation of infectious vectors on the winds of a cyclone.

The latest focus, the most unexpected, occurred this time east of the original core, on the island of Nevis during 2005-2006 [11]. The strain of the LY etiological agent on Nevis is very similar to that in Florida, and the hypothesis of a scenario identical to that at Cancun seems to be the most plausible. As Nevis is surrounded by numerous closely located islands, the Caribbean Arc southeast of Nevis, from Antigua-and-Barbuda to Trinidad, is worried about the possible spread of LY from North to South.

**Africa**

A LY type syndrome would appear to have been reported in Africa for the first time by Stein [12] in Tanzania. However, according to Schulling and Mpumani [13] the disease could have been present before 1900, that is, at the same time as the first reports of LY in the Caribbean. It was then described in Mozambique (Carvalho, 1958 quoted by Santana Quadros [14]) and Kenya [15, 16].

In West Africa, the first LYTS to be described would seem to have been in Nigeria, where it was called Awka disease [17]. At the beginning of the 1930s, some LYS were observed simultaneously in Togo (Kaincope disease), Ghana (Cape Saint Paul Wilt [CSPW]) [5] and in Cameroon (Kribi disease) [18, 19]. A LYS also exists in Equatorial Guinea but, curiously, Benin, which is separated from Togo by a river, has never been affected by this type of disease.

**Asia**

In Indonesia, lethal diseases of the coconut palm with frond yellowing exist on the island of Natuna (Natuna wilt) [20] and in Kalimantan (Kalimantan wilt) [21]. However, although phytoplasmas have been reported to be the etiological agents of these wilts, they do not belong to the 16S rDNA group containing the phytoplasmas of coconut LYTS in Africa and the Caribbean (Group 16S rDNA IV).

**Ecological diversity**

The LYTS of the Caribbean and Africa are all similar in their symptomatology. Yet, their environments differ considerably. The insect life and flora are variable. For instance, the LY vector in Florida, *Haplolixus crudus* – ex-*Myndus* – [22] does not exist in Africa. In addition, *H. crudus* has never been identified in Haiti or the Dominican Republic so far.

Another insect of the same family – *Cixiidae* – *Myndus adiopodoumeensis* – was long suspected of being the vector of CSPW in Ghana [23]. That species does not exist in Tanzania. As everyone can imagine, the flora on the northern coast of Zambezia is different from that on the northern coast of Jamaica or Yucatan. Consequently, the reservoirs of vectors and inoculum, be it native palms or any other plant, will probably be different.

**Phytoplasma diversity**

Phytoplasmas (e.g., MLO) are specifically associated with various LYTS in the Caribbean and Africa. They were firstly discovered under the electron microscope [25-30]. As phytoplasmas cannot currently be grown *in vitro*, it is impossible to demonstrate their etiological role in LYTS based on Koch’s postulates. However, the remission of some diseased palms has been obtained after treatment with tetracycline [31], which supports the thesis of a phytoplasma-based aetiology. With the development of molecular biology, polymerase chain reaction (PCR) has replaced lengthy searches under the microscope – fixing, embedding, cross-sections and examination under the microscope [32, 33].

Consequently, today, most phytoplasmosis diagnoses are carried out by PCR using DNA extracts from diseased coconut palms. The phytoplasma gene amplified by PCR is that of ribosomal RNA (rRNA). That gene is conserved, it is impossible to demonstrate their etiological role in LYTS based on Koch’s postulates. However, the remission of some diseased palms has been obtained after treatment with tetracycline [31], which supports the thesis of a phytoplasma-based aetiology. With the development of molecular biology, polymerase chain reaction (PCR) has replaced lengthy searches under the microscope – fixing, embedding, cross-sections and examination under the microscope [32, 33]. Consequently, today, most phytoplasmosis diagnostics are carried out by PCR using DNA extracts from diseased coconut palms. The phytoplasma gene amplified by PCR is that of ribosomal RNA (rRNA). That gene is conserved, and it is the one that has been most widely used to carry out all kinds of phylogeny and evolution studies [34-37].

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**Figure 1.** Specific host-vector-pathogen relations for diseases involving phloem-restricted micro-organisms transmitted by insect vectors.
Ribosomal RNA

As phytoplasmas are small wall-less bacteria (mollicutes), the sequence of their rRNA is fairly similar to that of bacteria, and the rRNAs of the various phytoplasmas causing several hundred syndromes display considerable sequence homology. However, an analysis of the PCR products by restriction fragment length polymorphism (RFLP) has led to the delimitation of 15 to 28 “groups” of rDNA – of phytoplasmas [37, 38]. Some of these different groups contain subgroups. For instance, group I would appear to contain 11 subgroups [38].

The phytoplasmas associated with LYS all belong to group IV called Coconut Lethal Yellows Group [37-39]. That group also contains some sequences of phytoplasmas from other palms, which, whilst not always displaying a “LYSY” (in particular, not yellowing but browning), are often considered to be species “susceptible to LY” (the LY disease in the Caribbean) (Howard and Harrison, [40]).

Group IV contains several subgroups (table 1). By studying RFLPs, Tymon [41] had already shown that the phytoplasmas of coconut palms in Africa were different from those in the Caribbean. Moreover, the phytoplasmas of Tanzania or Kenya were different from those of Ghana and Nigeria, which led to a distinction being made between at least three subgroups associated with the various LYS.

It should be noted that in this group, near the phytoplasmas associated with LY in the Caribbean, there is the phytoplasma associated with the Yucatan lethal decline (LDY) syndrome, which stands out well from that of LYS. A syndrome similar to that of LDY has been identified, associated with the same phytoplasma, on the Pacific coast of Mexico. It has been called coconut leaf yellowing to clearly distinguish its difference from that of LY.

Another phytoplasma, very close to that of LDY, has been identified in diseased coconut palms and Acrocomia aculeata (different syndrome from that of LY) in Honduras [42]. In addition, it has recently been shown that another subgroup exists that is associated with a coconut LY disease in the Dominican Republic [43] and a new subgroup associated with a disease assimilated to LY on Washingtonia robusta in Florida [44]. This new palm phytoplasma also exists in mixed infections with the LY phytoplasma in Phoenix dactylifera [44].

There, therefore, exist different phytoplasmas associated with different palm pathologies, involving or not involving a yellowing stage, and generally ending up more or less rapidly with a crownless stem.

Non-ribosomal sequences

Another sequence, not belonging to the ribosomal operon, is also used for PCR diagnosis of LY in Florida and the Caribbean. The PCR primers are known as LY/F/LYR [33]. However, those two primers do not give any amplification with certain LYS in Cuba or the Dominican Republic (unpublished results), or with coconut wilt diseases in the centre of Honduras [42]. This is further proof of the diversity of the phytoplasmas found in coconut.

Phytoplasma variability

A comparative analysis of the sequences obtained with the primers used to amplify the ribosomal sequence (P1/P7) reveals some highly conserved regions, notably in the 16S rRNA gene, and some more variable regions which correspond, among other things, to the spacer between the 16S and the tRNA\textsubscript{Ile} gene on the one hand, and between the tRNA gene and the 23S gene on the other hand (figure 2).

After alignment of the sequences specific to each strain (subgroup), some specific primers for PCR were constructed, to amplify a specific LYS. This was the case for primers G813/AKSR assumed to diagnose CSPW in Ghana [41]. We have used those primers in our research programme on CSPW, and we found that we sometimes obtained negative PCR’s for palms that were exhibiting typical CSPW symptoms. In order to find the origin of these false negatives, we sequenced the PCR products obtained with primers P1/P7 since primers G813/AKSR are inside the P1/P7 sequence (figure 2).

We found variability in the 16S/23S sequence of isolates collected in Ghana and those collected in Mozambique [45]. In all, for all the isolates, there were around 40 mutations in this sequence. It turns out that a mutation affects the sequence of a primer – AKSR – said to be specific to Ghana. Whilst the sequence of part 3’ of that primer is AAT\textsubscript{GTTG}, the sequence found in the majority of CSPW sequences is AAT\textsubscript{ATGG}. We therefore constructed a new primer with that sequence. It seems to enable the diagnosis of all CSPW cases [45].

In Ghana and Mozambique, LYS do not occur in the same way everywhere. For example, in Mozambique the disease in Zambezia province develops in large foci, in which from around 10 to 30 palms can be found at different stages of the disease. In Cabo Delgado province, further to the North, only small foci of three to four diseased or dying palms were found; the disease does not seem to have the same epidemic potential there as in Zambezia (unpublished).

Table 1. Subgroup 16S rDNA IV – Coconut Lethal Yellows group (according to [38]).

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Code</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut LY Ca. P. palmae</td>
<td>16s rIV – A</td>
<td>Florida</td>
</tr>
<tr>
<td>Yucatan coconut LY</td>
<td>16s rIV – B</td>
<td>Mexico</td>
</tr>
<tr>
<td>Lethal disease Tanzania</td>
<td>16s rIV – C</td>
<td>Tanzania</td>
</tr>
<tr>
<td>Cardulovica palmata yellowing</td>
<td>16s rIV – D</td>
<td>Mexico, Texas</td>
</tr>
<tr>
<td>Walnut witches broom Ca. P. castaneae</td>
<td>16s rIV – E</td>
<td>Korea</td>
</tr>
</tbody>
</table>

Figure 2. Regions of the ribosomal operon amplified by polymerase chain reaction (PCR) for the diagnosis.
An exhaustive study of the 16S/23S sequences in Ghana and Mozambique is currently under way and should soon enable us to specify this variability of the phytoplasmas associated with the LYTS in those two countries.

Variability for resistance
The diversity and variability of LYTS is also reflected in the resistance of coconut varieties to these different pathological syndromes. For instance, the Vanuatu Tall variety (VTT) – ex-New Hebrides Tall – is highly susceptible to LY in Jamaica, whereas the Malayan Yellow Dwarf (MYD) displayed a degree of resistance in the 1960s to 1980s [46]. In Ghana, after several trials were set up to test how coconut varieties reacted to CSPW between 1981 and 2007, it turned out that, on the contrary, the VTT is fairly resistant to CSPW and the MYD is highly susceptible [47]. In addition, although “Local Tall” coconut palms (West African Tall or WAT) be they from Ghana or Benin, are highly susceptible to CSPW, there are several “Local Tall” ecotypes (East African Tall) that display a degree of resistance to LDT (Mpunami, personal communication).

Conclusion
In the Caribbean, Africa and Asia, coconut plantings can be found that are affected by a yellowing, which can evolve more or less rapidly towards palm death. In most cases, such symptoms are reported as being cases of “LY”. However, coconut palms can display yellowing in response to various types of biotic or abiotic stress. For instance, a prolonged drought can cause nuts to fall, inflorescence necrosis and more or less marked yellowing. In the Caribbean zone, the same LY syndrome may be due to a trypanosome or a phytoplasma. In fact, most LYTS in the Caribbean and Africa are associated with the existence of phytoplasmas, and we speak of “LY”, as if the disease were one and the same. However, the term phytoplasma was created to designate all phloem-restricted mollicutes, transmitted by vector insects, and are impossible to grow in vitro [48]. There are at least 28 groups of phytoplasmas, and in those groups there are several subgroups which might be as many species; for example, four subgroups, four species possible for group 165 rx [37]. It can therefore be said that there exists a substantial diversity of LYTS and for each of those LYTS there is variability. It is important to take these data into account, particularly for PCR analysis, to search for vectors and for genetic control.

Acknowledgements. The authors benefited from the French Ministry of Foreign Affairs Priority Solidarity Fund project FSP No. 2004-34, “Agricultural research on tree and food crop cultivation in Ghana” (2005-2008).

REFERENCES
specifically primed polymerase chain reaction for the amplification of 16S rDNA. *Oléagineux* 1993; 48: 319-22.


